Electron Microscope Studies of Nuclear Extrusions in Pancreatic Acinar Cells of the Rat*

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PLATES 178 TO 181

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ABSTRACT

This paper describes "blebs" protruding from the surface of the nucleus into the cytoplasm. The "blebs" are separated from the cytoplasm by 2 membranes which are continuous with the outer and inner nuclear membranes. The "blebs" contain 3 structurally distinct substances. Two of these substances (β and γ substances) are similar to extranucleolar karyoplasm and nucleolar material. The other substance (α substance) is present in every "bleb," but it cannot be readily compared to a recognizable nuclear structure. Cytoplasmic vesicles are described that are apparently different from the Golgi vesicles or the vesicular component of the ergastoplasm. It is suggested that these vesicles may be of nuclear "bleb" origin.

A dark karyoplasmic zone extending from the region of the nucleolus into the nuclear "bleb" is shown. This zone may be similar in some respects to the preformed pathway ("*Leitbahn*") described by Altmann (3) and Hertl (28) and could reflect movement of nuclear material from the nucleolar region into the cytoplasm.

The "blebs" are thought to be homologous to structures described by many light microscopists, but they are considerably larger than the nuclear "blebs" described previously by electron microscopists.

The transfer of microscopically visible material from the nucleus into the cytoplasm has been described from time to time for many years and has been suggested as one of the possible mechanisms whereby the interphase nucleus could bring its influence to bear on cytoplasmic function. Observations described in this paper of vesicular structures, apparently budding out from the nucleus into the cytoplasm, provide further evidence of possible transfer of nuclear material into the cytoplasm. The size and morphology of these nuclear extrusions indicate that they correspond to the structures described by many light microscopists (3–5, 7, 8, 21, 27, 28, 30, 31, 33, 34, 39, 43, 51), but the extrusions seem to be much larger than those described previously by electron microscopists (1, 2, 6, 17, 23, 24, 37, 53).

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Materials and Methods

Tissues from 14 female Wistar rats weighing between 150 and 250 gm. were studied. Eight of the animals were fasted 24 to 48 hours before sacrifice and 6 were fed until the time of sacrifice. The animals were sacrificed by a blow on the head and small pieces of pancreas, generally no larger than 1 mm. in their greatest dimension, were fixed in Palade's buffered osmium tetroxide (38). After fixation at about 4°C. for 3 to 4 hours the tissues were dehydrated in a graded series of methanols. Tissues were embedded in a partially polymerized mixture (12:1) of *n*-butyl methacrylate and methyl methacrylate. Polymerization was completed overnight, at 60°C. Usually there were 5 to 8 suitable blocks of tissue from each animal available for study. In all animals the time which lapsed between the blow on the head and the immersion of pancreatic tissue into the fixative did not exceed 1 minute. The heart was still beating in each animal when the tissue was removed and no more than 10 seconds elapsed from the time the tissue was severed from its blood supply until it was in the fixative.

When the nuclear "blebs" were first observed, all blocks of tissue from each of the animals were sectioned and an attempt was made to survey the nuclei in multiple sections of each block for the presence of nuclear "blebs." In order to determine the incidence of "blebbing," sections from several blocks prepared from 12 animals were surveyed at a magnification of × 4,800. At least 1000 sections of nuclei were counted per animal and every "bleb" observed during the survey was photographed. Only those "blebs" that were attached to the nucleus and separated from the cytoplasm by a double membrane were counted. Counting of adjacent sections of nuclei was avoided in most instances so that 1000 nuclear sections usually represented 1000 different nuclei.

In the very thin sections that many investigators find ideal for electron microscope studies, the low contrast on the fluorescent screen makes it difficult to see the nuclear "blebs" and it is much easier to see these structures in relatively thick sections. Our sections were mounted on 100 and 200 mesh, formvar-coated copper screens, with or without carbon support. Some sections were treated for 5 minutes with $Pb(OH)_4$, according to Watson's method (49). An RCA-EMU-3C instrument was used.

OBSERVATIONS

The Nuclear "Bleb" Attached to The Nucleus.—In our observations a typical "bleb" of nuclear material is roughly circular in outline and is separated from the cytoplasm by two delicate membranes, which are obviously continuous with the outer and inner nuclear membranes (Figs. 1 to 3). The inner nuclear and inner "bleb" membranes occasionally appear to be doubled (Figs. 1 and 3). The "bleb" membranes show areas of apposition that are suggestive of "pore" formation. Ordinarily, the contents of the "bleb" are directly continuous with the main mass of karyoplasm. The narrow space between the outer and inner "bleb" membranes is continuous with the perinuclear space and is usually quite "clear" in electron micrographs (Figs. 1 to 3).

Within a typical "bleb" 3 structurally distinguishable substances may be identified which will be referred to as α, β, and γ substances. Such a designation is used only to facilitate discussion. The α substance is present in every "bleb" that has been photographed and it may be the only one of the 3 substances in a "bleb" (Figs. 1 to 3). It is uniformly distributed throughout the "bleb" and in low power micrographs appears as a homogeneous, amorphous material that is of relatively low density when compared with the main body of the nucleus (Fig. 2). At higher magnifications it is usually found to be composed of small granules and delicate filaments having a fine lacy pattern (Figs. 1, 3, and 4). The β substance consists of small granules and filaments that are denser than the α substance. The β substance is indistinguishable from the extranucleolar karyoplasm of the nucleus (Figs. 4 and 6). The γ substance is granular and is the densest of the 3 substances. It is similar to nucleolar material (Figs. 4 and 6).

The Detachment of the Nuclear "Bleb" and its Transference into the Cytoplasm.—The evidence that the nuclear "bleb" is detached from the nucleus and actually becomes "a part" of the cytoplasm is indirect and less convincing than the evidence for the formation of the primary "bleb." It is not difficult to find large, free cytoplasmic vesicles that are structurally identical to the "blebs" attached to nuclei (Figs. 2 and 4). Many of these structures, however, are in close topographic relationship to nuclei and might be attached to a nucleus at a level above or below the plane of section. Other vesicular structures that have structural features suggestive of nuclear "blebs" are quite often seen in the cytoplasm of cells. These vesicles may be found in cells with or without attached nuclear "blebs" and may be single or multiple (Fig. 5). Usually they are between 0.5 and 0.75 μ in diameter. Such vesicles are delimited by a membrane, which at times appears to be double and shows areas suggestive of "pore" formation. The contents of the vesicles are similar to the contents of nuclear "blebs." Even if these large cytoplasmic vesicles do not represent detached "blebs," their contents and membranes apparently distinguish them from the "clear" Golgi vesicles and the vesicular component of the ergastoplasm. These vesicles could be similar to the granule-packed, membrane-enclosed areas in sea urchin eggs, but these, of course, may also be of nuclear origin (2).

The Incidence of Nuclear "Blebbing."—Many of the animals forming the basis of this report were control animals from a study being conducted with Dr. Emmanuel Farber on the effects of ethionine on the fine structure of cells. Consequently, the experiments were not designed to produce violent alterations in the metabolic state of the pancreas and the nuclear "blebbing" of this report cannot be correlated with cytoplasmic function. However, some of the animals were fed and

others were fasted for 24 to 48 hours. The incidence of "blebbing" in these two groups has been compared. In fed animals 7.0 "blebs" per 1000 nuclear sections have been observed, while in the fasted group we have seen 5.3 "blebs" per 1000 nuclear sections; an insignificant difference (Table I). In both groups combined there is an average of 6.2 "blebs" per 1000 nuclear sections. The early observations led to the opinion that "blebbing" was more common in fed than fasted animals. This is apparently due to the fact that an occasional pancreatic lobule in the fed animals of this study has shown several "blebbed" nuclei. As many as 4 of 17 nuclei in one low power micrograph of a part of 1 lobule have shown "blebs." Some of our first observations were on such lobules giving the impression of a quite high incidence of "blebbing" in fed animals. This is not commonly observed, however, and one cannot conclude that this lobular distribution is correlated with the functional state of the pancreas.

The Possibility of Nuclear "Blebs" being Artifacts.—The observations reported here have not been reported by other authors, even though the mammalian pancreas has been extensively studied. This may be taken as an objection to the validity of these findings. In addition, good preservation of pancreatic tissue has not been achieved in all of our experiments and some of the "blebs" have been observed in cells of substandard quality. Opposed, however, to the possibility that the nuclear "blebs" are some peculiar artifact are the following observations. We have sectioned blocks at 7 levels and can see no relationship between poor preservation and "blebbing." "Blebs" may be seen in poorly preserved cells, but are also seen in cells which are well preserved. We have found "blebs" in every animal where at least 1000 nuclear sections were examined. Even in the one animal where no "blebs" were observed when counting 1000 nuclear sections (Table I), studies of other blocks following the statistical survey revealed "blebs" in this animal. Though only 1 strain of 1 species has been studied, the "blebs" have been observed in 5 different experiments using animals shipped at 4 different times. All of the animals have shown a normal weight gain before the experiments and no disease processes were observed at the time of sacrifice.

TABLE I

Animal Number	No. of "blebs"	No. of nuclear sections counted	Fasted or fed
1	4	1000	Fasted
2	0	1000	Fasted
3	13	1036	Fasted
4	2	1016	Fasted
5	9	1022	Fasted
6	4	1000	Fasted
7	2	1028	Fed
8	11	1000	Fed
9	5	1000	Fed
10	5	1000	Fed
11	8	1000	Fed
12	13	1000	Fed

Alterations in the Karyoplasm Associated with Nuclear "Blebbing."—In some of the micrographs the karyoplasm lying between the nucleolus and the nuclear "bleb" appears somewhat denser than other parts of the nucleus. This dense irregular band extends from the region of the nucleolus into the nuclear "bleb" (Fig. 6). In some instances the dark band in the karyoplasm is associated with a nucleolus consisting only of dark granules (Fig. 6). One gets the impression that the nucleolonema is absent; a hazardous interpretation in view of the strongly held concept that the nucleolonema is present in all stages of the cell cycle (19). The alterations in the karyoplasm and nucleolus have been seen only when α, β, and γ substances are present in the bleb.

DISCUSSION

Garnier, in 1899, studying a variety of glandular cells, suggested that modifications in nuclear size and shape indicate an active participation in the process of cellular secretion. He described movements of the "chromatic substance which seemed to originate in the nucleolus." The nuclear substance was said even to trespass the limits of the nuclear membranes and "expand in the form of a cloud into the neighboring cytoplasm" (21). In 1959, Hsu and Lou studying Cloudman melanoma cells and Wendt studying chick fibroblasts in tissue culture have beautifully illustrated nuclear extrusion by time lapse cinematography (30, 51). These are only 3 of a large number of papers which illustrate or suggest the transfer of nuclear material into the cytoplasm. There were such observations reported before Garnier's thesis (see Altmann (3), De Groot, La Gasse, and Sebruyns (17), Henry (27), Krabbe (31), and

Wilson (52) for some of the older literature) and many additional studies have been reported during the 60 year interval between the work of Garnier and Hsu and Lou and Wendt. Various aspects of the nucleocytoplasmic relationship have been covered in recent reviews (Baud (7), Bloch (10), Briggs and King (14), Gates (22), Hertl (28), Kurnick (32), Mazia (36), Stich (44), and Vincent (47)), and no attempt will be made here to review the subject. The admirable report of Hertl (28) carefully compares many of the opinions about the structure and function of the nucleolus (nucleolar apparatus) and discusses the transfer of nuclear and nucleolar material into the cytoplasm.

Most morphological studies of nucleocytoplasmic interrelationships indicate that some substance, visible with the light microscope, is transferred from the nucleus to the cytoplasm. At least 3 anatomic mechanisms of such extrusions have been described. (*a*) A nuclear vesicle ("bleb" or "pocket") is said to form at the nuclear membrane and the "bleb" then ruptures or is dispersed in some fashion into the cytoplasm (7, 30, 31, 51). (*b*) The nuclear membrane is described as opening temporarily ("*Schleusenmechanismus*" of Berg) and such openings are associated with transfer of nuclear material into the cytoplasm (3–5, 8). (*c*) Lastly, the nuclear material is said to diffuse across an intact nuclear membrane (28). According to Hertl the majority of investigators adhere to the latter concept. In addition, Lettré (34) and others have described the extrusion of whole nucleoli into the cytoplasm and this phenomenon has been frequently described during oögenesis (see Brachet (11) for recent literature).

Other investigators have presented opinions as to the functional significance of nuclear extrusion. Henry (27) and Pawlikowski (39) suggest that nuclear extrusion proceeds and even initiates cytoplasmic activity or secretion. Altmann (3) has presented structural forms of the nucleus said to represent a functional cycle ("*Funktionsformwechsel*") of the nucleus of the exocrine cells of the mouse pancreas, which can be correlated with a "functional cycle" of the cytoplasm. Schiller thinks that the extrusion of nuclear material is the beginning of amitotic cell division (43).

There have also been cytochemical and biochemical studies that suggest the transfer of nuclear material into the cytoplasm and in some respects these experiments tend to parallel the morphological studies (Brachet (12, 13), Caspersson and Schultz (16), Ficq (20), and Marshak (35)).

In spite of the wealth of electron microscope studies of many cells and tissues in recent years, the evidence in electron micrographs supporting the concept of extrusion of nuclear material into the cytoplasm has been meager. "Pores" that would permit passage of relatively large particles have been described by Callan and Tomlin (15). Watson has studied nuclear pores extensively, has discussed the possible relationship between the double nuclear membranes and cytoplasmic membranes, and has reviewed the pertinent literature on this subject (48, 50). Similar "pores" have also been shown clearly by Horstmann and Knoop (29). De Robertis has discussed the passage of granules through pores of the nuclear membranes of neurons (18). However, the presence of "true pores" in the nuclear membrane has been questioned. Policard and Collet (40) and Haguenau and Bernhard (26) have suggested that the "pores" are usually closed by a single delicate membrane. According to their view, persistent anatomic continuity between the cytoplasm and karyoplasm through "true pores" would seem to be the exception rather than the rule.

The studies of Afzelius (1, 2) and Rebhun (41, 42) of oöcytes of the sea urchin, snail, and clam have indicated that "annulate" membranes of the cytoplasm are derived from nuclear membranes. Similar structures have also been described by Swift ("annulate lamellae") in amphibian pancreatic acinar cells and rat spermatids (45). Direct extrusion of Feulgen-negative nuclear material into the cytoplasm has been illustrated by Anderson and Beams in the ovarian nurse cells of the reduvid bug, *Rhodnius prolixus* (6). Gay (23, 24) and Moses (37) have shown "nuclear blebbing" in *Drosophila* salivary gland and crayfish spermatocytes respectively. Wischnitzer has shown quite clearly "nuclear membrane evagination" in amphibian oöcytes (53). The "blebs" described by these authors (6, 23, 24, 37, 53) appear to be delimited by a single rather than a double membrane. De Groot, La Gasse, and Sebruyns have described in electron micrographs nuclear vesicles said to go into the cytoplasm of the hepatic cells of the albino rat, but their published micrograph does not show the structures clearly (17). Much of the ultrastructural evidence for transfer of nuclear material into the cytoplasm has been reviewed in the valuable papers of Bern-

hard (9), Haguenau (25), Briggs and King (14), and Swift (45).

One is impressed by two points when comparing the light microscope with the electron microscope evidence that suggests the transfer of nuclear material into the cytoplasm. First, the nuclear "blebs," membranous structures, and extruded material of these electron microscope studies are almost beyond the limit of resolution of the light microscope; and secondly, the majority of these reports deal with non-mammalian tissue and frequently with germ cells. The "pores" described by Afzelius (1, 2), Rebhun (41, 42), and Swift (45) are certainly "submicroscopic." The "blebs" of Gay (23, 24), Moses (37), and Wischnitzer (53) barely exceed 0.5 μ and are usually smaller. Even though Moses has published a light micrograph of a Feulgen reaction of a nucleus that shows "blebbing," he used a magnification of $\times$ 3,700 to show the "blebs" and the picture would be difficult to interpret without the accompanying, distinct electron micrograph. Gay, in her studies of the salivary gland of the larvae of *Drosophila melanogaster*, has demonstrated nuclear "blebs" that persist after a variety of fixatives; and has correlated their presence with the accumulation of intracellular secretion granules (23, 24). She has specifically suggested that the process could be of importance in the formation of secretion granules. Her observations are invaluable but she states that the "blebs" are only "discernible at the level of resolution afforded by the electron microscope." It is, therefore, doubtful whether the structures thus far described by electron microscopists are the counterparts of Garnier's "cloud of nuclear material" expanding into the cytoplasm; and it is equally doubtful whether they are likely to be equivalent to the nuclear extrusions demonstrated by Hsu and Lou (30), Lettré (34), Wendt (51), and others who have photographed structures several microns in diameter.

In addition, the light microscopists have described nuclear extrusions in a wide variety of mammalian tissues and the pancreatic acinar cells have frequently been described as showing the phenomenon. Even though the mammalian pancreas has been a favorite object of study by electron microscopists, there has as yet been no description, to my knowledge, of structures that can be readily compared to the nuclear extrusions observed with the light microscope. The "blebs" described in this paper seem to correspond fairly well with the nuclear structures observed by light microscopy. In fact, there is a striking similarity between our micrographs and the pictures published by Hsu and Lou (30), Lettré (34), and Wendt (51). The observations of Hsu and Lou suggest that several small "secondary pockets" form on a "primary pocket" and rupture into the cytoplasm a few minutes after being formed. In view of their brief duration it is not to be expected that many such "secondary pockets" will be demonstrated by the methods of electron microscopy, but one, morphologically compatible with a "secondary pocket," was photographed in the course of this study.

Altmann (3) and Hertl (28) have emphasized the presence of a preformed pathway ("*Leitbahn*") between the region of the nucleolus and the nuclear membrane which is traversed by Feulgen-positive granules passing from the region of the nucleolus to the nuclear membrane. The alterations in the karyoplasm thought to exist in some of the micrographs of this study could be comparable to the findings of these authors (Fig. 6). However, we have no evidence, by the methods of electron microscopy, for the existence of such a pathway in a nucleus that does not show "blebbing."

Conclusions as to the chemical nature of the nuclear "blebs" are not justified from these studies. The β substance in the attached "bleb" is indistinguishable from the extranucleolar karyoplasm and the γ substance is similar to nucleolar material. Consequently, it is difficult to refrain from suggesting that the β substance could be rich in DNA and the γ substance rich in RNA. Even if these substances had been shown to be present in the "blebs," the static methods of this study by no means prove that the "blebs" are actually detached and become "a part" of the cytoplasm.

The nuclear "blebs" reported here could be a peculiar artifact, evidence of amitotic cell division, or could indicate the transfer of nuclear material into the cytoplasm. The latter interpretation is preferred, but is presented with some reservations. Our studies do not suggest that nuclear "blebbing" can be correlated with cytoplasmic function. The observations of others, however, suggest a correlation between "blebbing" and cellular function. Before Hsu and Lou could consistently demonstrate "blebbing," it was necessary to put their cultures in fresh media (30). Laird *et al.* (33) have described a paranuclear vacuole in hepatic cells during refeeding, after a period of starvation, that could be homologous to

the "blebs" discussed in this paper (46). The observations of Altmann, Gay, and others that suggest a correlation between nuclear "blebbing" and cellular function have been mentioned. All such evidence for a relationship between nuclear extrusion and cellular function is only suggestive, but the data which have accumulated are quantitatively impressive. The observations reported here possibly indicate that the phenomenon of nuclear "blebbing" occurs in the living pancreatic acinar cells of the rat; and suggest once more that the periodic transfer of relatively large amounts of "formed" nuclear material into the cytoplasm may be a normal feature of cell activity.

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Bibliography

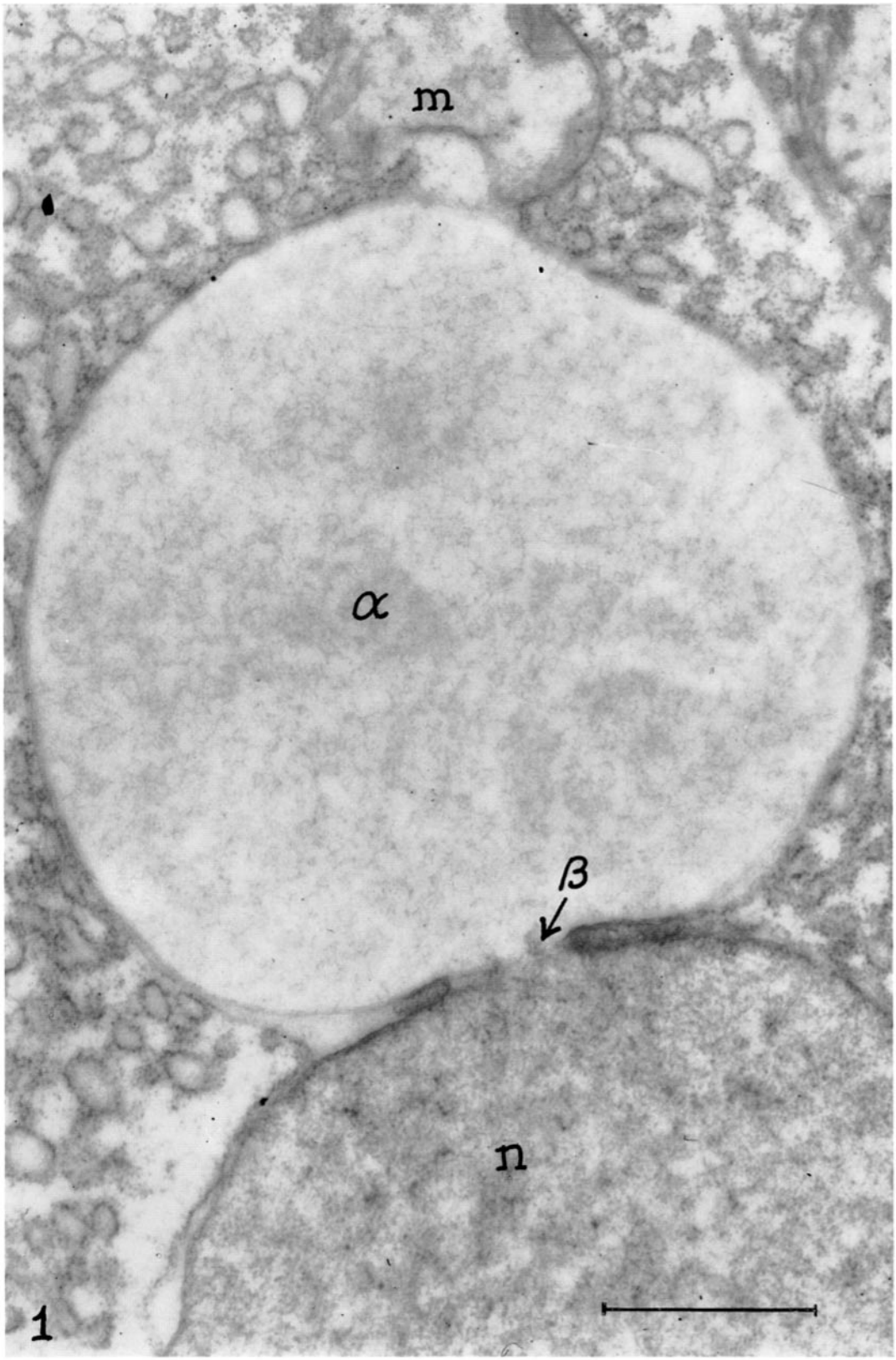
1. Afzelius, B. A., Electron microscopy of Golgi elements in sea urchin eggs, *Exp. Cell Research*, 1956, **11,** 67.
2. Afzelius, B. A., Electron microscopy on the basophilic structures of the sea urchin eggs, *Z. Zellforsch. u. mikr. Anat.*, 1957, **45,** 660.
3. Altmann, H. W., Über den Funktionsformwechsel des Kernes im exokrinen Gewebe des Pancreas, *Z. Krebsforsch.*, 1952, **58,** 632.
4. Altmann, H. W., Zur Morphologie der Wechselwirkung von Kern und Cytoplasma, *Klin. Woch.*, 1955, 306.
5. Altmann, H. W., Über die Abgabe von Kernstoffen in das Protoplasma der menschlichen Leberzelle, *Z. Naturforsch.*, 1949, **4,** 138.
6. Anderson, E., and Beams, H. W., Evidence from electron micrographs for the passage of material through pores of the nuclear membrane, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 439.
7. Baud, C. A., Recherches sur la structure de la membrane nucléaire, *Acta Anat.*, 1953, **17,** 113.
8. Berg, W., Über den Übertritt von Kernstoffen in das Cytoplasma, *Z. Mikr.-Anat. Forsch.*, 1932, **28,** 565.
9. Bernhard, W., Ultrastructural aspects of nucleocytoplasmic relationship, *Exp. Cell Research*, 1958, suppl., **6,** 17.
10. Bloch, D. P., Changes in the desoxyribonucleoprotein complex during the cell cycle, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 113.
11. Brachet, J., Biochemical Cytology, New York, Academic Press, Inc., 1957, 129.
12. Brachet, J., La détection histochimique des acides pentose nucléiques, *Comp. rend. Soc. biol.*, 1940, **133,** 88.
13. Brachet, J., La localization des acides pentosenucléiques, perdant le développment des amphibiens, *Comp. rend. Soc. biol.*, 1940, **133,** 90.
14. Briggs, R., and King, T. J., Nucleocytoplasmic interactions in eggs and embryos, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1959, **1,** 537.
15. Callan, H. G., and Tomlin, S. G., Experimental studies on amphibian oöcyte nuclei, I. Investigation of the structure of the nuclear membrane by means of the electron microscope, *Proc. Roy. Soc. London, Series B*, 1950, **137,** 367.
16. Caspersson, T., and Schultz, J., Ribonucleic acids in both nucleus and cytoplasm and the function of the nucleolus, *Proc. Nat. Acad. Sc.*, 1940, **26,** 507.
17. DeGroot, M., La Gasse, A., Sebruyns, M., La "sécretion nucléaire" vue au microscope électronique, *Cellule*, 1956, **58,** 55.
18. De Robertis, E., The nucleo-cytoplasmic relationship and the basophilic substance (ergastoplasm) of nerve cells (electron microscope observations), *J. Histochem. and Cytochem.*, 1954, **2,** 341.
19. Estable, C., and Sotelo, J. R., The behavior of the nucleolonema during mitosis, *in* Fine Structure of Cells, New York, Interscience Publishers, Inc., 1955, 170.
20. Ficq, A., Étude autoradiographique du métabolisme des protéines et des acides nucleiques au cours de l'oogénese chez les batraciens, *Exp. Cell Research*, 1955, **9,** 286.
21. Garnier, C., Contribution à l'étude de la structure et du fonctionnement des cellules glandulaires sécreuses, Du rôle de l'ergastoplasme dans la sécrétion, Thesis, L'Université de Nancy, No. 50, cited by Haguenau, F., *Internat. Rev. Cytol.*, 1958, **7,** 426.
22. Gates, R. R., Nucleoli and related nuclear structures, *Bot. Rev.*, 1942, **8,** 337.
23. Gay, H., Chromosome-nuclear membrane-cytoplasmic interrelations in *Drosophila*, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 407.
24. Gay, H., Nucleo-cytoplasmic relations in salivary-gland cells of *Drosophila*, *Proc. Nat. Acad. Sc.*, 1955, **41,** 370.
25. Haguenau, F., The ergastoplasm: Its history, ultrastructure and biochemistry, *Internat. Rev. Cytol.*, 1958, **7,** 425.
26. Haguenau, F., and Bernhard, W.; Particularites structurales de la membrane nucléaire. Étude au microscope electronique de cellules normales et cancereuses, *Bull. Assn. Franc. Étude Cancer*, 1955, **42,** 537.

27. Henry. A., Étude histologique de la fonction sécrétoire de l'épididyme chez les vertébrés supérieurs, *Arch. anat. micr.*, 1900, **3,** 229.
28. Hertl, M., Zum Nucleolus-Problem, *Z. Zellforsch. u. mikr. Anat.*, 1957, **46,** 18.
29. Horstmann, E., and Knoop, A., Zur Struktur des Nucleolus und des Kernes, *Z. Zellforsch. u. mikr. Anat.*, 1957, **46,** 100.
30. Hsu, T. C., and Lou, T. Y., Nuclear extrusion in cells of Cloudman melanoma *in vitro*, *in* Pigment Cell Biology, (M. Gordon, editor), New York, Academic Press, Inc., 1959, 315.
31. Krabbe, K. H., Histologische und emkryologische Untersuchungen über die Zirbeldrüse des Menschen, *Anat. Hefte*, 1916, **54,** 189.
32. Kurnick, N. B., Histochemistry of nucleic acids, *Internat. Rev. Cytol.*, 1955, **4,** 221.
33. Laird, A. K., Barton, A. D., and Nygaard, O., Synthesis of protein and ribonucleic acid in rat liver during refeeding after starvation, *Exp. Cell Research*, 1955, **9,** 523.
34. Lettré, R., Observations on the behavior of the nucleolus of cells *in vitro*, *in* Fine Structure of Cells, New York, Interscience Publishers, Inc., 1955, 141.
35. Marshak, A., Evidence for nuclear precursor of ribo- and desoxyribonucleic acid, *J. Cell and Comp. Physiol.*, 1948, **32,** 381.
36. Mazia, D., Physiology of the cell nucleus, *in* Trends in Physiology and Biochemistry, (E. S. G. Barron, editor), New York, Academic Press, Inc., 1952, 77.
37. Moses, M. J., Studies on nuclei using correlated cytochemical, light, and electron microscope techniques, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 397.
38. Palade, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95,** 285.
39. Pawlikowski, T., Sur les altérations morphologiques du noyau des cellules chromaffines, *Bull. Histol. Appl.*, 1938, **15,** 149.
40. Policard, A., et Collet, A., Apropos due problem des pores nucleàires, *Bull. micr. Appl.*, 1958, **8,** 74.
41. Rebhun, L. I., Electron microscopy of basophilic structures of some invertebrate oocytes, I. Periodic lamellae and the nuclear envelope, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 93.
42. Rebhun, L. I., Electron microscopy of basophilic structures of some invertebrate oocytes, II. Fine structure of the yolk nuclei, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 159.
43. Schiller, E., Kerneinschlüsse and Amitose, *Z. Zellforsch. u. mikr. Anat.*, 1949, **34,** 356.
44. Stich, von H., Bau und Funktion der Nukleolen, *Experientia*, 1956, **12,** 7.
45. Swift, H., The fine structure of annulate lamellae, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 415.
46. Swift, H., personal communication, July, 1959.
47. Vincent, W. S., Structure and chemistry of nucleoli, *Internat. Rev. Cytol.*, 1955, **4,** 269.
48. Watson, M. L., The nuclear envelope. Its structure and relation to cytoplasmic membranes, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 257.
49. Watson, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Applications of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 727.
50. Watson, M. L., Further observations on the nuclear envelope of the animal cell, *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 147.
51. Wendt, E., Lebendbeobactungen an bestrahlten Interphasekernen, *Z. Zellforsch. u. mikr. Anat.*, 1959, **49,** 677.
52. Wilson, E. B., The Cell in Development and Heredity, New York, The Macmillan Company, 1928, 91.
53. Wischnitzer, S., An electron microscope study of the nuclear envelope of amphibian oocytes, *J. Ultrastruct. Research*, 1958, **1,** 201.

Explanation of Plates

Plate 178

Fig. 1. The central portion of the micrograph is occupied by a large "bleb." It is separated from the cytoplasm by a double membrane and these membranes are continuous with the inner and outer nuclear membranes. The inner nuclear and inner "bleb" membranes sometimes appear as if doubled. Except for a small fragment of β substance at the base of the "bleb," it is entirely filled with α substance. Even at this magnification the "lacy" pattern of the α substance is apparent. *n*, nucleus, *m*, mitochondrion, α, α substance, β, β substance. × 34,000.

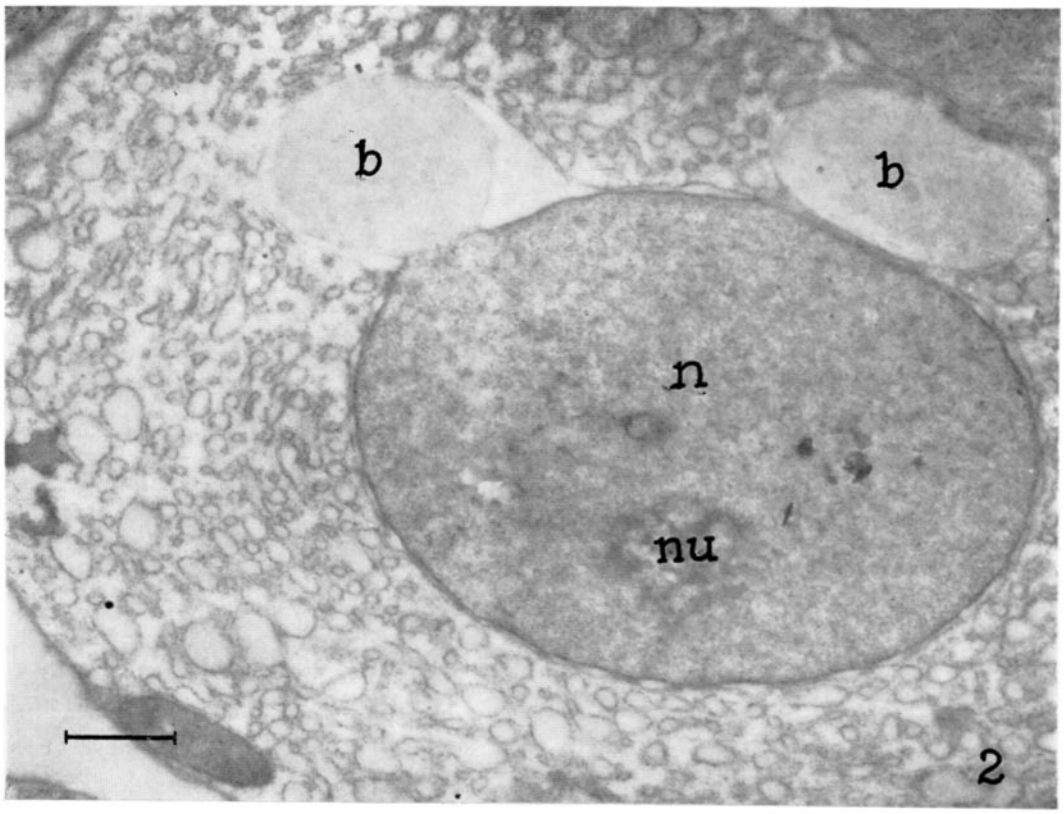


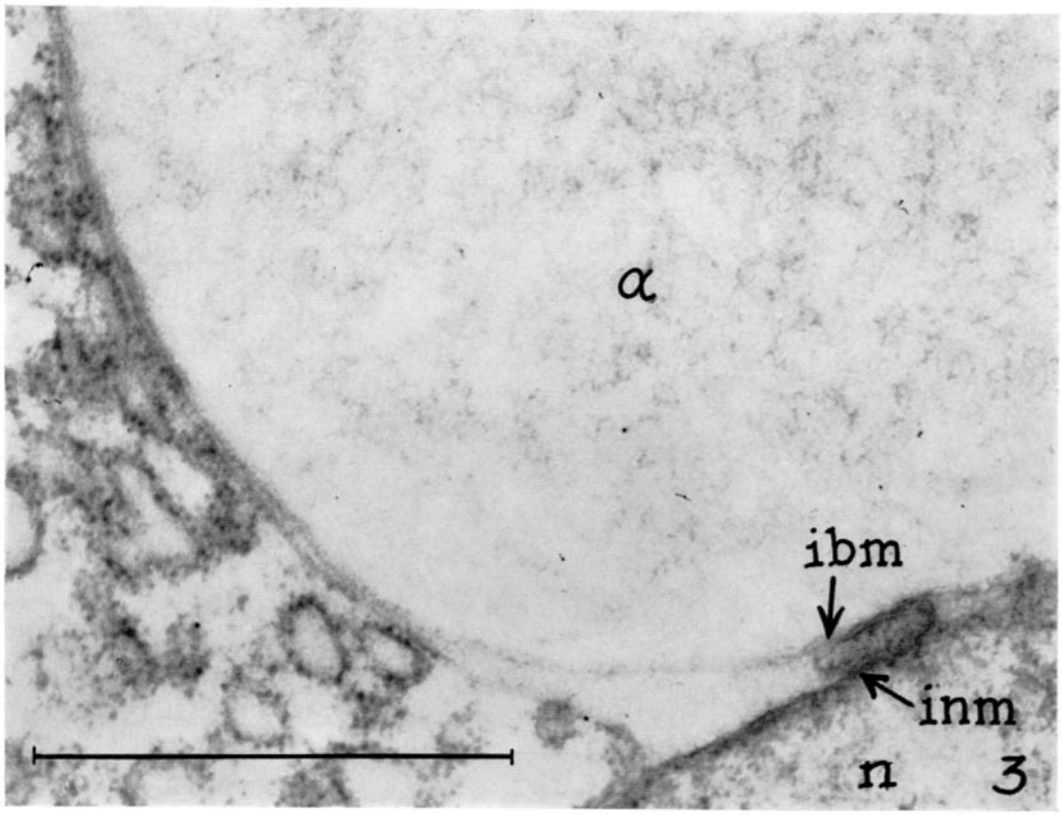
(Clark, Jr. Nuclear extrusions in pancreatic acinar cells)

PLATE 179

FIG. 2. A binucleate cell, which presents two "blebs," is shown. The "bleb" at the left is attached to the nucleus, while the "bleb" at the right is not attached in the section plane of the micrograph. Both "blebs" contain only α substance. On the left it is easy to see that the perinuclear space is continuous with the "peri-bleb" space. *n*, nucleus, *b*, "bleb," *nu*, nucleolus. × 14,000.

FIG. 3. An enlargement of a part of Fig. 1. The "lacy" pattern of the α substance in the "bleb" is shown. The suggestion of doubling of the inner "bleb" membrane may be seen where the inner nuclear membrane turns outward to form the inner "bleb" membrane. *n*, nucleus, α, α substance, *ibm*, inner "bleb" membrane, *inm*, inner nuclear membrane × 63,500.



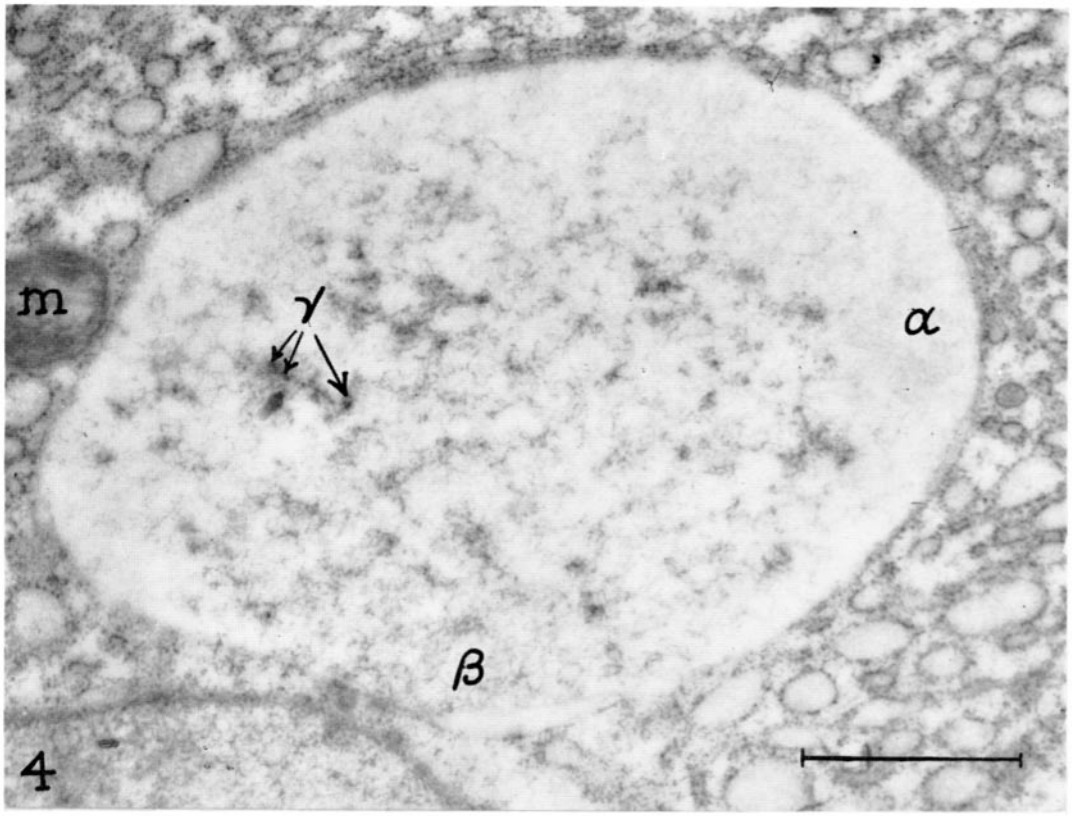


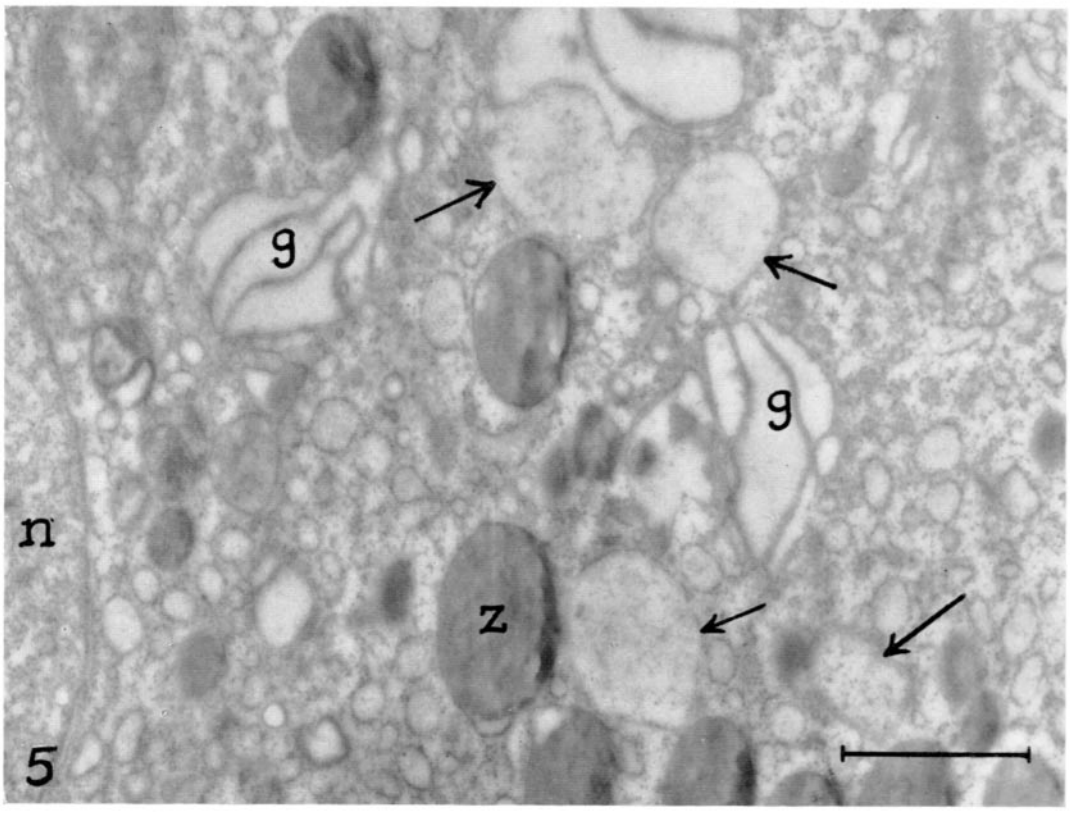
(Clark, Jr.: Nuclear extrusions in pancreatic acinar cells)

PLATE 180

FIG. 4. A "detached bleb," near a nucleus, is shown. The "bleb" contains α, β, and γ substances. The section was treated with $Pb(OH)_4$ for 5 minutes (49). *n*, nucleus, α, α substance, β, β substance, γ, γ substance, *m*, mitochondrion. × 29,000.

FIG. 5. The cytoplasmic vesicles shown at the arrows are suggestive of "bleb" origin. This cell did not show a "bleb" attached to the nucleus in this section plane. *n*, nucleus, *g*, Golgi zone, *z*, zymogen granule. × 24,500.

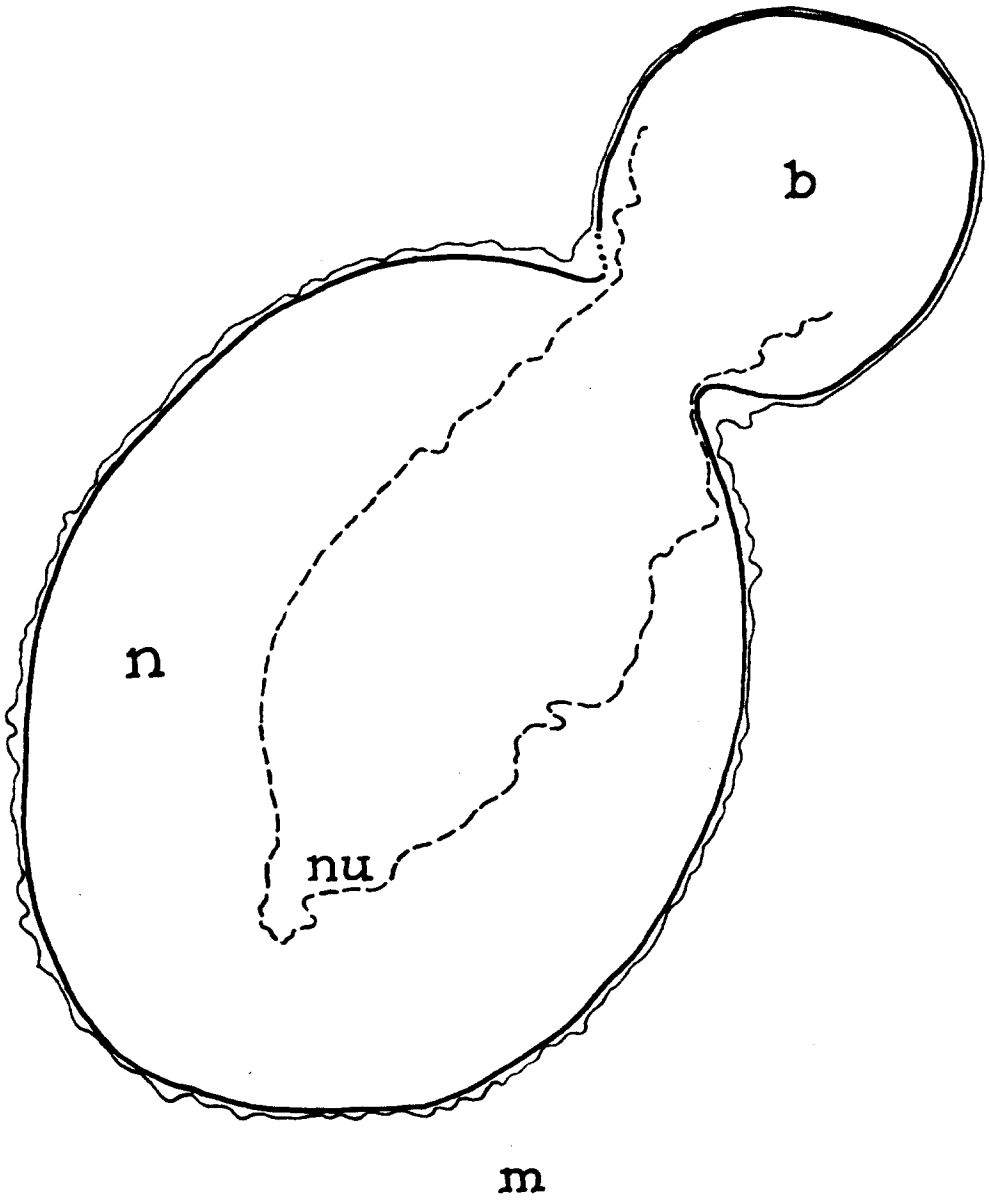




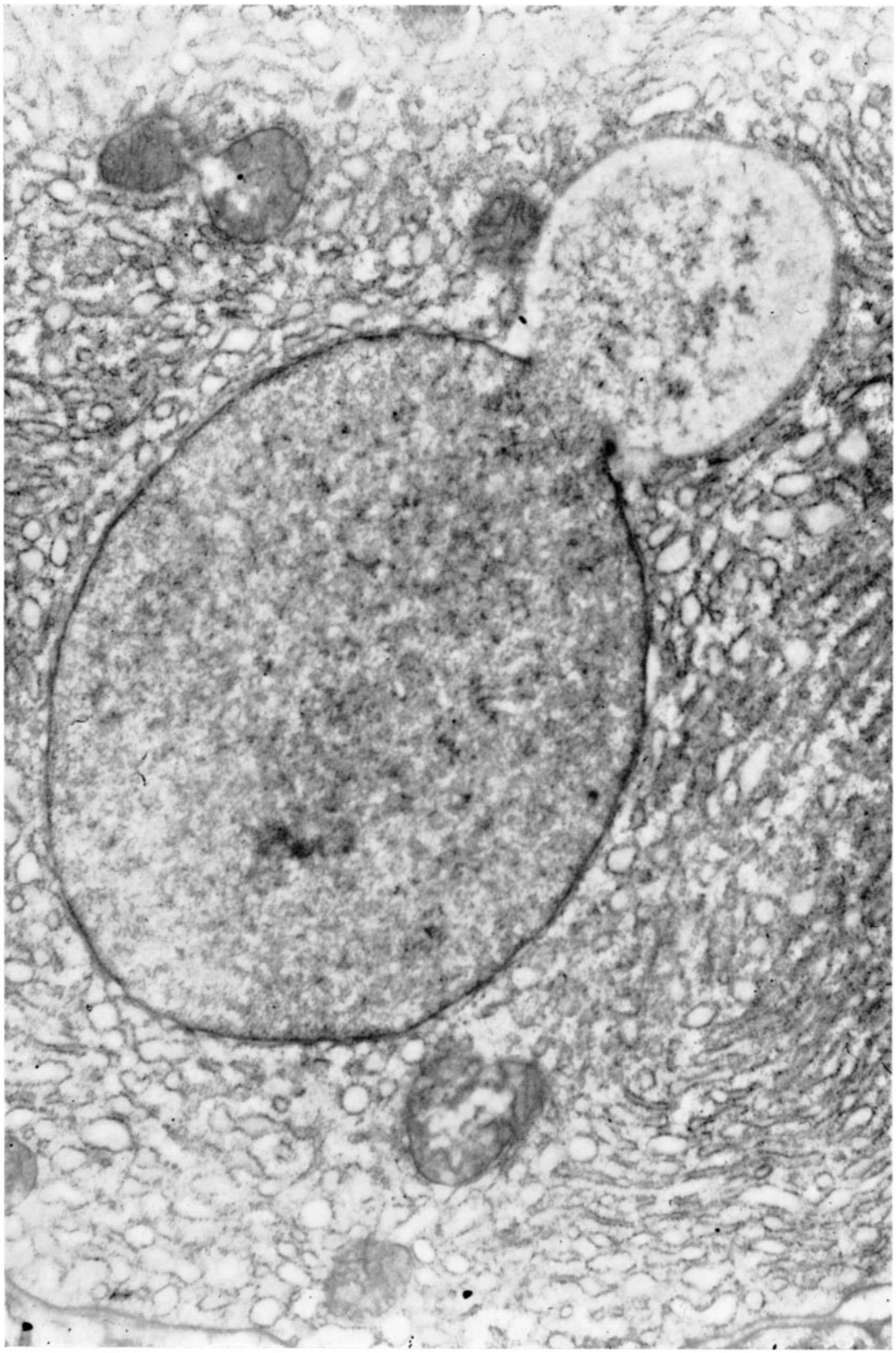
(Clark, Jr.: Nuclear extrusions in pancreatic acinar cells)

PLATE 181

FIG. 6. The micrograph shows a "bleb" containing α, β, and γ substances. The overlay shows the outline of a darker karyoplasmic zone that is thought to represent an alteration sometimes associated with "blebbing." The dark granular area (*nu*) is interpreted as the nucleolus. In this micrograph there is no suggestion of a nucleolonema. *n*, nucleus, *b*, bleb, *m*, mitochondrion. × 19,500.



6



(Clark, Jr.: Nuclear extrusions in pancreatic acinar cells)